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A Comparison of Two Different Doses of Ultraviolet-A Light Exposure on Nitric Oxide Production

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Abstract

Purpose: The present study investigated different doses of ultraviolet-A (UV-A) light on plasma nitric oxide metabolites and cardiorespiratory variables.

Methods: Ten healthy male participants completed three experimental conditions, 7 days apart. Participants were exposed to no light (CON); 10 J•cm^2 (15 min) of UV-A light (UVA10) and 20 J•cm^2 (30 min) of UV-A light (UVA20) in a randomized order. Plasma nitrite [NO_2^-] and nitrate [NO_3^-] concentrations, blood pressure (BP), and heart rate (HR) were recorded before, immediately after exposure and 30 min post-exposure. Whole-body oxygen utilization ($\dot{V}O_2$) and skin temperature were recorded continuously.

Results: None of the measured parameters changed significantly during CON (all P>0.05). $\dot{V}O_2$ was significantly reduced immediately after UVA10 (P=0.03) despite no change in plasma [NO_2^-] (P>0.05). Immediately after exposure to UVA20, plasma [NO_2^-] was higher (P=0.014) and $\dot{V}O_2$ tended to be lower compared to baseline (P=0.06). There were no differences in [NO_2^-] or $\dot{V}O_2$ at the 30 min time-point in any condition. UV-A exposure did not alter systolic BP (SBP), diastolic BP or MAP (all P>0.05). UV-A light did not alter plasma [NO_3^-] at any time point (all P>0.05).

Conclusions: This study demonstrates that a UV-A dose of 20 J•cm^2 is necessary to increase plasma [NO_2^-] although a smaller dose is capable of reducing $\dot{V}O_2$ at rest. Exposure to UV-A did not significantly reduce BP in this cohort of healthy adults. These data suggest that exposure to sunlight has a meaningful acute impact on metabolic function.

Key Words: Sunlight, Nitrate, Nitrite, Blood Pressure, metabolic rate

Abbreviations

Analysis of variance: ANOVA

Blood pressure (BP)

Centimetres: cm

Control: (CON)

Degrees Celcius: ^{o}C

Degrees North: ^{o}N

Delta: Δ

Diastolic BP: DBP

Heart rate: HR

Intravenous: I.V.

Joules per centimetre squared: $J \bullet cm^2$

Kilograms: kg

Litre: L

Mean arterial BP: MAP

Microliter: µL

Micromolar: µM

Millimolar: mM

Minutes: Min

Nanometers: nm

Nanomolar: nM

Nitrate: NO_3^-

Nitric Oxide: NO

Nitric Oxide Synthases: NOS

Nitrite: NO_2^-

Number: n

Oxygen utilization: $\dot{V}O_2$

Sodium hydroxide: NaOH

Standard error of the mean: SEM

Systolic BP: SBP

Total nitrosated products: RXNO

Ultraviolet- UV

Ultraviolet-A: (UV-A)

Ultraviolet Radiation: UVR

Weighted volume: w/v

Xanthinine oxioreductases: XOR

Zinc Sulphate: $ZnSO_4$

10 J•cm^2: (UVA10)

20 J•cm^2: (UVA20)

Introduction

Nitric oxide (NO) is produced by NO synthases (NOS) from the oxidation of L-arginine and acts as a multifunctional signaling molecule that regulates a number of key biological processes including neuronal signaling, immune function, mitochondrial respiration, and vascular tone (Carr and Ferguson 1990; Moncada and Higgs 1991; Ignarro 2002). NO is rapidly oxidized within the human body to nitrite (NO_2^-) and nitrate (NO_3^-), with the latter accounting for the majority of NO-derived compounds within the body. Recent evidence suggests that NO_3^- and NO_2^- are important reservoirs of NO that act independently of NOS activity (Lundberg et al. 2009, 2015). Although NO_3^- is considered to be physiologically inert, it can be reduced to biologically active NO_2^- by bacteria in the oral cavity (Duncan et al. 1995) and gut (Tiso and Schechter 2015) or reduced by xanthinine oxidoreductases (XOR) (Lundberg et al. 2009). NO_2^- can initiate physiological effects or be reduced further to NO under hypoxic (Castello et al. 2006) and acidic (Modin et al. 2001) conditions.

Several studies have demonstrated that increasing plasma levels of NO_3^- and NO_2^- through dietary NO_3^- ingestion and via sunlight exposure can reduce blood pressure (BP) (Larsen et al. 2006; Webb et al. 2008; Opländer et al. 2009; Kapil et al. 2010; Vanhatalo et al. 2010; Siervo et al. 2013; Wylie et al. 2013; Muggeridge et al. 2015; McIlvenna et al. 2017). Increasing the circulating concentration of NO metabolites has also been shown to elicit other cardiovascular and metabolic effects, including the reduction of resting oxygen utilization ($\dot{V}O_2$) (Larsen et al. 2014; Whitfield et al. 2016). Intriguingly, both dermis and epidermis contain NO_2^- at concentrations substantially greater than those in blood (Paunel et al. 2005). Exposing the skin to light in the UV-A wavelength range (315-400nm) leads to photodecomposition of these NO derivatives and release of NO into the circulation with consequent biological effects (Paunel et

al. 2005; Mowbray et al. 2009; Suschek et al. 2010). UV-A induced NO production has been shown to be independent of NOS, temperature, and vitamin D status (Liu et al. 2014). Given that basal plasma [NO_2^-] is suggested to be a marker of endothelial function (Kleinbongard et al. 2006), whereby low levels correlate with increased cardiovascular risk (Allen et al. 2009), UV-A induced NO production could conceivably modulate cardiovascular homeostasis.

Despite the potential health significance of these observations, little is known about the minimal dose of UV-A exposure required to alter circulating NO levels. Whole body UV-A exposure of 20 J•cm^2 (equivalent to ~30 min of Mediterranean summer sunlight) has been consistently shown to mobilize NO_2^- from the skin into the plasma (Liu et al. 2014) and reduce systemic BP (Opländer et al. 2009; Liu et al. 2014; Muggeridge et al. 2015). To the best of our knowledge, this has been the only dose of UV-A used in human volunteer studies related to the mobilization of NO from skin. However, it has been shown *in vitro* that UV-A light of 9 J•cm^2 causes a dose-dependent increase in NO production in isolated keratinocytes without causing DNA damage (Holliman et al. 2017). This may be important as current public health messages favor sun avoidance due to the established carcinogenic effects of habitual UV exposure (Kennedy et al. 2003; Rigel 2008). Given that exposure to sunlight may impact positively on markers of cardiovascular health, further research is warranted to determine the minimum dose of UV-A exposure to elicit these effects.

Therefore, the aim of the present study was to compare the effects of two different doses of UV-A light on plasma [NO_2^-], [NO_3^-], BP and resting $\dot{V}O_2$. We hypothesized that UV-A light would increase plasma NO derivatives and reduce MAP and resting $\dot{V}O_2$ in a dose-dependent manner.

2 Methods

2.1. Participants

Ten healthy males (age 28 ± 5 years, stature 180 ± 9 cm, body mass 80.8 ± 11.0 kg) volunteered to participate in the study following ethical approval by the School of Science and Sport Ethics Committee at the University of the West of Scotland. Participants self-determined their skin type via the Fitzpatrick Skin Questionnaire (Fitzpatrick 1988) as skin type 2 (n = 1) and skin type 3 (n = 9). Written informed consent was obtained from all participants prior to the commencement of the study. All participants were non-smokers, apparently healthy, were not regular users of anti-bacterial mouthwash, and reported no use of medication. Prior to each trial participants were instructed to avoid prolonged sunlight exposure and caffeine on the morning of each visit and to avoid NO_3^- rich foods (such as beetroot and lettuce), high intensity exercise, and alcohol consumption within 48 h of each trial.

2.2. Experimental design

Each participant attended the laboratory on three separate occasions and was exposed to either no light (control, CON), 10 J•cm^2 of UV-A light (UVA10), or 20 J•cm^2 of UV-A light (UVA20), in a randomized counter-balanced order. Each condition was separated by 7 days and performed at the same time of day for each participant. Trials were performed in Scotland at 55.78^{o}N latitude between July and December. Each trial was conducted in the morning (before 11 am) after an overnight fast and following the consumption of ~500 ml of bottled water upon awakening. Dietary and exercise habits were self-recorded via a 48 h recall at the beginning of visit one and repeated on each visit thereafter. Compliance to these factors was determined at the beginning of each visit.

2.3. UV-A Exposure

Whole body UV-A exposure was delivered using a commercially available UV-A light therapy system (Waldmann, UV302 L, Germany). UV-A light occurs naturally in the 315-400 nm wavelength band, and the present system emitted light at wavelengths between 315 and 351 nm. In all conditions, participants lay supine on a medical plinth for the duration of the experiment and wore shorts and protective glasses. Participants were instructed to close their eyes for duration of the light exposures. During CON, the UV-A system remained off. The trials were conducted in a temperature-controlled laboratory (22 ± 1.3^{o}C). The dose of UV-A was automatically calculated based on exposure time and calibrated irradiance at a distance of 21 cm from the abdominal skin. Exposure time was 15 min in UVA10 and 30 min in UVA20. Calibration of the UV-A light source was conducted as per the manufacturer's instructions.

2.4. Experimental procedures

On arrival, stature and body mass were recorded following a bladder void. Upon lying supine for a period of 30 min prior to baseline measurements, an I.V. catheter was inserted into the antecubital vein of each participant for collection of venous blood samples. In the UVA10 condition, participants lay supine for an additional 15 min prior to baseline measurements in order to time-match the duration of the experiment across all three conditions. Skin temperature was continuously monitored (Squirrel SQ2022, Cambridge, England) via thermistors placed at four anatomical sites (triceps, chest, quadriceps, and calf). Participants were fitted with a heart rate (HR) monitor (Polar Electro, Oy, Finland), which was continuously monitored by telemetry. Resting $\dot{V}O_2$ was measured via indirect calorimetry using breath-by-breath analysis

(Medgraphics, Milan, Italy). The volume measurement of the system was calibrated prior to each trial using a 3 L syringe as per the manufacturers' instructions. Gas analyser calibration was performed with two gases of known concentrations (calibration gas: 5% carbon dioxide, 12% oxygen and nitrogen balance and reference gas: 21% oxygen and nitrogen balance) (Air Liquide Healthcare, Belgium). Resting $\dot{V}O_2$ data were taken as an average for 20 min at baseline, during treatment, and for 30 min post-exposure. Upper and lower limits of agreement were calculated for resting $\dot{V}O_2$, and data points exceeding two standard deviations from the mean were excluded from analysis. Brachial BP was measured in triplicate with 1 min between measures at baseline, immediately after light exposure (0 min), and 30 min after exposure using a manual stethoscope and sphygmomanometer (Accoson, London, UK), with the pressure cuff placed at the upper part of the non-dominant arm positioned at the level of the right atrium. Mean values were used for analysis. Mean arterial BP (MAP) was calculated using the following equation: MAP = [(2 x diastolic pressure + systolic) / 3]. Following each BP measure, 8 ml of venous blood was collected from the opposite arm at baseline, 0 min, and 30 min post-exposure. Blood was collected in vacutainers containing EDTA and immediately centrifuged at 4000 rpm at 4^{o}C for 10 min (Harrier 18/80, MSE, UK). Plasma was extracted and immediately stored at -80^{o}C for later analysis of [NO_3^-] and [NO_2^-]. Intravenous lines were flushed with 2 ml of 0.9% saline solution immediately following each blood draw.

2.5. Analysis of Plasma NO Metabolites

Gas-phase chemiluminescence was used to determine plasma [NO_3^-] and [NO_2^-], as previously described (Pinder et al. 2008; Muggeridge et al. 2014; McIlvenna et al. 2017). Briefly, following the creation of a standard curve at several concentrations (0 to 1000nM for NO_2^-) and (0 to 100µM for NO_3^-), samples were thawed in a water bath at 37^{o}C for 3 min. Plasma

samples were injected in duplicate into a customized enclosed purge vessel containing the respective reagent mixture. For the determination of NO_2^-, a reagent containing 1% sodium iodide in 4 ml of glacial acetic acid kept at 50^{o}C was used to reduce NO_2^- to NO.

Before the determination of NO_3^-, plasma samples were deproteinized using zinc sulfate ($ZnSO_4$) and sodium hydroxide (NaOH). Samples were made up in 1 ml aliquots (200uL plasma: 400µL $ZnSO_4$ (10% w/v) and 400 µL NaOH solution (200 µL de-ionized water: 200 µL 1M NaOH) and vortexed for 30 s. Samples were then centrifuged at 4000 rpm for 5 min and supernatants were used for NO_3^- analysis. For the determination of NO_3^-, a reagent containing 32 mg of vanadium trichloride (VCl_3), 4 ml of 1M hydrochloric acid, and 500 µl of de-ionized water was used in a sealed purge vessel maintained at 95^{o}C. NO was quantified using an NO analyzer (Sievers NOA 280; Analytix, UK). The area under the curve (AUC) was then used to determine concentrations by plotting the standard curve and dividing the AUC of each sample by the gradient of the slope. The co-efficient of variance (COV) for both plasma $[NO_2^-]$ and $[NO_3^-]$ was better than 5%.

2.6 Data Analysis

The distribution of the data were tested using the Shapiro-Wilk test. A two-way repeated-measures ANOVA was used to examine the differences between 'condition', 'time' and the 'condition x time' interaction for all variables. Post-hoc analysis of the significant main effects were conducted using paired t-tests and adjusted for multiple comparisons using the Bonferroni correction. Statistical significance was accepted at $P < 0.05$. The inclusion of 95% CI for mean differences are presented with *P* values and effect sizes (Cohen's D), when appropriate. Effect sizes were interpreted as: small effect > 0.2; medium effect > 0.5; large effect > 0.8. Data were

analyzed using SPSS (Version 22.0) and Graph Pad Prism (Version 7.02). Data in the text are presented as mean ± standard deviation. Data in figures are presented as group delta (Δ) ± standard error of the mean (SEM) relative to pre-treatment baselines.

3.1 Results

3.1.1 NO Metabolites

Plasma [NO_2^-] changes are shown in Fig 1A. Plasma [NO_2^-] was not different between the three conditions at baseline (CON 109 ± 54 nM, UVA10 108 ± 55 nM, UVA20 162 ± 75 nM, P = 0.18). There was a significant main effect of 'condition' (P = 0.004), 'time' (P = 0.001) and 'condition x time' interaction (P < 0.001) on plasma [NO_2^-]. Following UVA20, plasma [NO_2^-] was increased from baseline at the 0 min time point (95% CI, 25.7 to 216.9; P = 0.014; d = 1) but was not different at 30 min (P = 1.0; d = 0.2). Plasma [NO_2^-] did not differ from baseline at the 0 min or the 30 min time points in both the CON and UVA10 trials (all P > 0.4, d < 0.3).

Changes in plasma [NO_3^-] are shown in Fig 1B. Plasma [NO_3^-] was different between the three conditions at baseline (CON 45.6 ± 16.2 µM, UVA10 55.1 ± 21.9 µM, UVA20 39.5 ± 16.8 µM, P = 0.04). Post hoc comparisons revealed baseline plasma [NO_3^-] was higher in UVA10 compared to UVA20 (95% CI, 0.4 to 31; P = 0.05; d = 1), but there was no difference between CON and UVA10 (P = 0.4) or CON and UVA20 (P = 0.9). There was a significant main effect for 'condition' (P = 0.013) on plasma [NO_3^-]. Overall, plasma [NO_3^-] was significantly higher in UVA10 when compared to UVA20 (95% CI, 2.5 to 25.6; P = 0.02; d = 1), but was not different to CON (P = 0.7). There was no significant main effect for 'time' (P = 0.7) or 'condition x time' interaction (P = 0.109) for plasma [NO_3^-].

3.1.2 Blood Pressure, Heart Rate and Skin Temperature

There was no significant 'condition x time' interaction for systolic BP (SBP) (P = 0.91; Table 1), diastolic BP (DBP) (P = 0.19; Table 1) or MAP (P = 0.27; Figure 1C; Table 1). There was no difference in BP variables at baseline (P > 0.5; Table 1). Although no significant difference was observed in BP, effect sizes indicate that following UVA20, DBP reduced to a small extent immediately following (P = 0.3; d = 0.3) and 30 min (P = 0.15; d = 0.3) post-exposure. After UVA20, there was a small reduction in MAP immediately following exposure (P = 0.3; d = 0.3) and a moderate decrease 30 min after exposure (P = 0.1; d = 0.5) when compared to baseline (Table 1). Skin temperature and HR data are shown in Table 1. There was no significant main effect of 'condition x time' for HR (P = 0.5). There was a trend towards a 'condition x time' interaction for skin temperature (P = 0.06). Effect sizes show a small increase in skin temperature immediately following UVA20 (d = 0.4), but no difference between any other time points (d < 0.2).

3.1.3 Resting $\dot{V}O_2$

There was a significant 'condition x time' effect on resting $\dot{V}O_2$ (P = 0.047; Fig 1D). Resting $\dot{V}O_2$ was not different between the three conditions at baseline (CON 273 ± 37 ml/min^{-1}, UVA10 267 ± 32 ml/min^{-1}, UVA20 269 ± 37 ml/min^{-1}; P= 0.8). Following UVA20, there was a trend for a reduction in resting $\dot{V}O_2$ at 0 min compared to the baseline (95% CI, -0.759 to 36.1, P = 0.061; d = 0.4), but values were not different at 30 min (P = 1; d < 0.2). Following UVA10, resting $\dot{V}O_2$ was significantly reduced at 0 min (95% CI, 0.837 to 16.3; P = 0.03; d = 0.3) but did not differ from baseline 30 min after exposure (P = 1; d < 0.2). In CON, resting $\dot{V}O_2$ did not change at 0 min or 30 min (both P = 1; d < 0.2).

4.1 Discussion

Exposure to 20 J•cm^2 of UV-A light, a dose equivalent to approximately 30 min of Mediterranean summer sunlight (Diffey 2002), has previously been shown to increase plasma [NO_2^-] and lead to a sustained reduction in BP (Opländer et al. 2009; Liu et al. 2014). The present study explored the effects of different doses of UV-A exposure and the effects on circulating NO metabolites, BP, and resting $\dot{V}O_2$. The principal findings were that 20 J•cm^2 of UV-A exposure resulted in a brief, but significant increase in plasma [NO_2^-] whereas 10 J•cm^2 was insufficient to alter the concentration of this NO metabolite. In contrast to previous findings, exposure to UV-A light in either dose did not alter BP. However, we here demonstrate for the first time that exposure to UV-A light reduces resting $\dot{V}O_2$. While these data suggest that a minimum dose of 20 J•cm^2 is necessary to augment plasma NO availability, further work is required to better understand the therapeutic effects of UV-A light on cardiovascular and metabolic health.

4.1.1 Dose-Dependent Effects of UV-A Light on NO Metabolites

The observed increase in plasma [NO_2^-] following 20 J•cm^2 but not 10 J•cm^2 (~ 15 min of Mediterranean summer sunlight, or 40 min of British summer sunlight) of UV-A light supports our original hypothesis that the UV-induced release of NO metabolites from the skin is dose dependent. We demonstrate that 20 J•cm^2 of UV-A light increased plasma [NO_2^-] by 75% immediately following cessation of exposure, which is higher than the proportional increases of 45% (Opländer et al. 2009) and 40% (Liu et al. 2014) that have been previously reported. On the other hand, the absolute increase in plasma [NO_2^-] was higher (~200 nM) in the study by Liu and colleagues (2014) was higher than in the present study (123 nM), Collectively, the

present study and other studies in the area (Opländer et al. 2009; Liu et al. 2014; Muggeridge et al. 2015) suggest that UV-A induced NO production may alter the overall circulating pool of NO. However, it is clear that there is profound inter-individual variability of NO_2^- following UV-A challenge (Opländer et al. 2009). This may be explained by recent data from Holliman and colleagues (2017) who demonstrated diverging baseline levels of skin NO content in their skin donors and a variable magnitude of NO release from isolated keratinocytes in response to UV-A exposure. Both age and body composition are known to influence the storage and release of NO metabolites from the skin (Ma et al. 2015). These variables, along with skin type and habitual sunlight exposure may help explain the divergent response to UV-A between individuals and study cohorts. The total storage of NO metabolites in the skin is likely to be important as it has recently been demonstrated *in vitro* that NO_2^- is converted to NO upon UV-A exposure (Holliman et al. 2017).

In contrast to previous findings (Opländer et al. 2009; Liu et al. 2014), the observed increase in plasma [NO_2^-] immediately following exposure with 20 J•cm^2, was not sustained 30 min post-exposure. One possible explanation is that the source and delivery method for the UV-A light differed between all of these studies involving human volunteers. We speculate that the overall 'dose' or intensity of light may contribute to diverging NO_2^- release and overall NO kinetics. Previous studies have utilized the same dose of 20 J•cm^2 of UV-A light (Opländer et al. 2009; Liu et al. 2014; Muggeridge et al. 2015), however over different time periods and intensities. In the present study, the intensity of light was the same in UVA10 and UVA20, where exposure time was manipulated to alter the overall dose. I*n vitro* experiments highlight the relevance of the UV-A source showing that overall NO kinetics are altered depending on the distance from the UV-A light source (Dejam et al. 2003) and potentially the specific wavelength of the light. The middle to long UV-A wavelength range of 340-400 nm has been

shown to be the major contributor to overall NO production in isolated keratinocytes in response to UV-A challenge (Holliman et al. 2017). In the present study, our UV-A light source emitted its maximum intensity at 351 nm.

In contrast with previous research, plasma [NO_3^-] was not altered by UV-A exposure. Although we found no change in this variable, we speculate that the study was underpowered to see pronounced effects of UV-A exposure on plasma [NO_3^-], particularly in the first 30 mins. Liu and colleagues (2014) have previously demonstrated that 20 J•cm^2 of UV-A light significantly reduced [NO_3^-]. The authors suggested that UV-A exposure may enhance the enzymatic conversion of NO_3^- to NO_2^- and that there may be some direct photolysis of NO_3^- that yields NO. Future research is warranted to elucidate the role of UV-A induced alterations in plasma NO_3^-. Our data supports the notion that NO_2^- is more rapidly altered following UV-A challenge.

4.1.2 Blood Pressure is not altered by UV-A light

Our study demonstrated that UV-A light did not significantly alter BP. This finding contrasted our study hypothesis and previous research which has demonstrated that 20 J•cm^2 effectively reduces BP in a healthy cohort (Opländer et al. 2009; Liu et al. 2014). Nevertheless, there was a moderate reduction in MAP (d = 0.5) 30 min after exposure in the UV20 condition with a concomitant moderate reduction in heart rate (d = 0.5). It should be highlighted that the small sample of participants in this study were all young, healthy, and normotensive males. The most likely explanation for the absence of a significant reduction in BP was that the elevation in plasma [NO_2^-] was not sustained long enough to elicit a pronounced biological effect.

The experimental procedures may also have limited the extent to which UV-A light may have reduced BP. Specifically, the measurement of BP following the treatments in each condition was preceded by a 1 h period of lying supine. The consequence is that this sustained period of lying supine likely induced postural venodilation (Gemignani et al. 2008) and lowered BP prior to the experimental intervention. Indeed, DBP was 70 ± 7 mmHg and MAP was 85 ± 6 mmHg at baseline across all three conditions. This may have limited any further biologically significant reductions in BP following UV-A exposure. Recently, we have shown that posture and the period of time of lying supine can alter both BP and plasma [NO_2^-] (Liddle et al. 2017), which emphasizes that posture should be carefully considered when conducting future research in this area. Although it has been suggested that even small reductions in BP are protective against cardiovascular incidence (Lawes et al. 2004), the clinical and biological significance of the small reductions in this study are unclear.

4.1.3 UV-A Light Reduces $\dot{V}O_2$

A notable finding in this study is that $\dot{V}O_2$ fell during both light exposures. To our knowledge, this study is the first to explore the effects of UV-A light on resting metabolism in humans. A significant reduction in resting $\dot{V}O_2$ was observed despite no elevation in plasma [NO_2^-] during UVA10, whereas a trend for a reduction was found in the presence of a significant elevation in plasma [NO_2^-] in UVA20. While the mechanisms accounting for the reduction in $\dot{V}O_2$ could not be ascertained in the present study, we speculate that the complexity of NO conversion and appearance from NO_2^- or other nitrogen oxides following UV-A challenge may have accounted for this finding. We hypothesized that resting $\dot{V}O_2$ would be reduced in the presence of elevated NO_2^- as others have shown that NO_2^- inhibited respiration by ~60% when applied to primary skeletal myotubes, *in vitro* (Larsen et al. 2011). Others have demonstrated that increasing plasma [NO_2^-] and NO bioavailability via dietary NO_3^- supplementation reduces $\dot{V}O_2$ at rest

(Larsen et al. 2014; Whitfield et al. 2016). Larsen and colleagues (2014) speculated that the reduction in resting metabolic rate was most likely due to an NO-mediated inhibition of cytochrome *c* oxidase (Carr and Ferguson 1990) and found that changes in this parameter following dietary NO_3^- were independent of insulin sensitivity and thyroid hormones. However, Whitfield and Colleagues (2016) observed a similar reduction in whole body $\dot{V}O_2$ at rest, in the absence of change in skeletal muscle mitochondrial respiration. It is conceivable that the changes in resting $\dot{V}O_2$ observed in the present study following UV-A may have occurred via other NO-related mechanisms involving species distinct from those of the canonical NO_3^-- NO_2^- - NO pathway. It must also be considered that the absence of an elevation in plasma NO_2^- following UVA10 and presence in UVA20 may be indicative of an NO-independent mechanism.

The reduction in $\dot{V}O_2$ following UV-A exposure appears to be transient as values returned to baseline 30 min after exposure in both light exposure conditions. The biological consequences of a sustained reduction in resting $\dot{V}O_2$ following UV-A exposure are unclear but must be considered as contraindicative for energy balance. This is of relevance given the composition of UV-A in overall sunlight exposure is approximately 90% (Diffey 2002). Furthermore, UV exposure has been proposed as a potential intervention for obesity and cardiometabolic dysfunction (Geldenhuys et al. 2014; Fleury et al. 2016), potentially via vitamin D or NO mediated effects (Gorman et al. 2017). Although the precise mechanism of the UV-A induced reduction in $\dot{V}O_2$ are unknown, the present study further highlights that sunlight exposure may have a potentially confounding impact on key markers of cardiometabolic health, which should be considered in epidemiological research.

4.1.4 Limitations

The present study is not without limitations. Firstly, the study lacked ecological validity in the sense that acute exposure to artificial UV-A light in the laboratory tells us little about the relevance of environmental sunlight exposure for habitual vascular homeostasis. Secondly, while our data suggests there is a minimum dose of UV-A light exposure that is required to elicit meaningful increases in NO bioavailability, the existence of a dose-response relationship cannot be ascertained without more extensive investigations. Although we observed an increase in plasma [NO_2^-] following 30 min of simulated sunlight exposure, we did not assess its release from skin stores and are therefore unable to provide a comprehensive assessment of the kinetic changes in NO derivatives and release from the skin. Such data are necessary to truly understand the role of the skin in the regulation of NO bioavailability and to establish whether UV-A exposure may be cardioprotective.

5.1 Conclusions

The present study is the first to determine that at least 20 J•cm^2 of UV-A exposure (~30 min of Mediterranean summer sunshine) is required to significantly increase plasma [NO_2^-] in healthy human participants. Surprisingly, elevations in plasma NO availability were not met with a significant reduction in BP, which has been consistently observed in previous studies. These contrasting data may relate to experimental differences in UV-A exposure protocols or inter-individual variability in responses between participants. Although BP was not reduced in this study, UV-A exposure in doses of either 10 or 20 J•cm^2 reduced resting $\dot{V}O_2$. These data provide further evidence to suggest that environmental exposures of sunlight may have a meaningful impact on cardiovascular and metabolic function.

Figure 1: A Plasma [NO_2^-]; B Plasma [NO_3^-]; C Mean arterial pressure; and D resting oxygen utilization ($\dot{V}O_2$), D following no exposure (CON•), 10 J•cm^2 (UVA10 ▪), and 20 J•cm^2 (UVA20▲) of UV-A light (group delta (Δ) mean ± SEM). Shaded area signifies the period of UV-A light exposure in UVA10 and UVA20. * Denotes significant increase in plasma NO_2^- compared to baseline.

6.1 References

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